

Amendments to the Specification

Please add the following new paragraph on page 1, line 1, as follows:

The present application is a divisional application of United States patent application number 09/967,301 filed September 28, 2001, which claims priority to patent application number 0109858.1 filed in Great Britain on April 23, 2001, the entire disclosures of which are hereby incorporated by reference.

Please replace the paragraph on page 6, lines 4-6, with the following amended paragraph:

Additionally, the present invention provides a fluorescent protein derived from Green Fluorescent Protein (GFP) and having the amino acid sequence as set forth in ~~SEQ ID No. 3~~SEQ ID NO: 3 of Figure 3.

Please replace the paragraph on page 6, lines 8-10, with the following amended paragraph:

The present invention also provides a fluorescent protein derived from Green Fluorescent Protein (GFP) and having the amino acid sequence as set forth in ~~SEQ ID No. 4~~SEQ ID NO: 4 of Figure 4.

Please replace the paragraph on page 8, lines 19-20, with the following amended paragraph:

Figure 1 is the nucleotide Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5) and referred to herein as ~~SEQ ID No. 1~~SEQ ID NO: 1.

Please replace the paragraph on page 8, lines 21-22, with the following amended paragraph:

Figure 2 is the corresponding amino acid sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5) and referred to herein as ~~SEQ ID No. 2~~SEQ ID NO: 2.

Please replace the paragraph on page 8, lines 23-24, with the following amended paragraph:

Figure 3 is the predicted amino acid sequence of F64L-S175G-E222G-GFP and referred to herein as ~~SEQ ID No. 3~~SEQ ID NO: 3.

Please replace the paragraph on page 9, lines 1-2, with the following amended paragraph:

Figure 4 is the predicted amino acid sequence of F64L-S65T-S175G-GFP and referred to herein as ~~SEQ ID No. 4~~SEQ ID NO: 4.

Please replace the paragraph on page 10, lines 14-17, with the following amended paragraph:

Suitably, the amino acid F at position 64 may be substituted by an amino acid selected from the group consisting of ~~L, I, V, A and G~~Leu, Ile, Val, Ala and Gly, thereby providing F64L, F64I, F64V, F64A, or F64G substitutions. In a preferred embodiment of the first aspect, the amino acid ~~F~~Phe is substituted by ~~L~~Leu at position 64.

Please replace the paragraph on page 10, lines 19-22, with the following amended paragraph:

Suitably, the amino acid S at position 175 may be substituted by an amino acid selected from the group consisting of ~~G, A, L, I and T~~Gly, Ala, Leu, Ile and Thr, thereby providing S175G, S175A, S175L, S175I and S175T substitutions. In a preferred embodiment of the first aspect, the amino acid ~~S~~Ser is substituted by ~~G~~Gly at position 175.

Please replace the paragraph on page 11, lines 1-5, with the following amended paragraph:

In embodiments where the amino acid S at position 65 is substituted, it is suitably substituted by an amino acid selected from the group consisting of ~~G, A, L, C, V, I and T~~Gly, Ala, Leu, Cys, Val, Ile and Thr, thereby providing S65G, S65A, S65L, S65C, S65V, S65I or S65T substitutions. Preferably, the amino acid substitution at position 65 is the S65T substitution.

Please replace the paragraph on page 11, lines 7-11, with the following amended paragraph:

In embodiments where the amino acid ~~E~~Glu at position 222 is substituted, it is suitably substituted by an amino acid selected from the group consisting of ~~G, A, V, L, I, F, S, T, N and Q~~Gly, Ala, Val, Leu, Ile, Phe, Ser, Thr, Asn and Gln, thereby providing E222G, E222A, E222V, E222L, E222I, E222F, E222S, E222T, E222N or E222Q

substitutions. Preferably, the amino acid substitution at position 222 is the E222G substitution.

Please replace the paragraph on page 11, lines 18-21, with the following amended paragraph:

Preferably, the fluorescent protein according to the first aspect has an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein having the sequence: ~~SEQ ID No. 2~~SEQ ID NO: 2.

Please replace the paragraph on page 11, line 23, through page 12, line 3, with the following amended paragraph:

A preferred protein according to the present invention is a protein in which, in relation to ~~SEQ ID No. 2~~SEQ ID NO: 2 of GFP, the amino acid ~~F~~PPhe at position 64 has been substituted by ~~L~~LLeu, the amino acid ~~S~~SSer at position 175 has been substituted by ~~G~~GGly and the amino acid ~~E~~EGlu at position 222 has been substituted by ~~G~~GGly, and is shown herein as having the amino acid sequence as set forth in ~~SEQ ID No. 3~~SEQ ID NO: 3.

Please replace the paragraph on page 12, lines 5-9, with the following amended paragraph:

An alternative preferred protein according to the present invention is a protein in which, in relation to ~~SEQ ID No. 2~~SEQ ID NO: 2 of GFP, the amino acid ~~F~~PPhe at position

64 has been substituted by ~~L~~Leu, the amino acid ~~S~~Ser at position 65 has been substituted by ~~F~~Thr and the amino acid ~~S~~Ser at position 175 has been substituted by ~~G~~Gly, and is shown herein as having the amino acid sequence as set forth in ~~SEQ ID No. 4~~SEQ ID NO: 4.

Please replace the paragraph on page 12, lines 11-16, with the following amended paragraph:

Suitably, the GFP or functional GFP-analogue used to derive the fluorescent protein may be obtained from any convenient source. For example, native GFP derived from species of the genus *Aequorea*, suitably *Aequorea victoria*. The chromophore in wtGFP from *Aequorea victoria* is at positions 65-67 of the predicted primary amino acid sequence (~~SEQ ID No. 2~~SEQ ID NO: 2). In a preferred embodiment, the GFP is derived from *Aequorea victoria*.

Please replace the paragraph on page 12, line 18, through page 13, line 11, with the following amended paragraph:

The modified proteins of the present invention may be produced by introducing mutations in a sequence of the nucleic acid that encodes the protein. As used herein, a preferred sequence of the gene encoding wtGFP is derived from *Aequorea victoria*, published by Chalfie et al, (Science, (1994), 263, 802-5) disclosed as ~~SEQ ID No. 1~~SEQ ID NO: 1 (Figure 1). The corresponding amino acid sequence is shown in ~~SEQ ID No. 2~~SEQ ID NO: 2 (Figure 2). Alternative sequences of the GFP gene may be used, for example, the nucleotide (and predicted amino acid) sequences of the GFP gene described

by Prasher et al, (Gene (1992), 111, 229) and the sequences as disclosed in WO 97/11094. In addition, alternative gene sequences that encode the fluorescent protein may incorporate a consensus Kozak nucleotide sequence (Kozak, M., Cell (1986), 44, 283), or preferred mammalian codons, to provide improved translation in mammalian systems. The nucleotide sequence corresponding to the fluorescent protein may also encode useful restriction enzyme sites and additional elements such as target sites for enzymes and purification tags. Methods for incorporation of a Kozak region, preferred mammalian codons, restriction enzyme sites, enzyme sites and purification tags are well known in the art and may result in the incorporation of amino acid residues and a change in numbering of amino acid residues in the fluorescent protein relative to the wtGFP numbering in the sequence provided.

Please replace the paragraph on page 15, lines 1-4, with the following amended paragraph:

Preferably, the nucleic acid molecule according to the third aspect encodes a fluorescent protein having an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein having the sequence: ~~SEQ ID No. 2~~SEQ ID NO: 2.

Please replace the paragraph on page 15, lines 14-16, with the following amended paragraph:

Preferably, the nucleic acid molecule encodes a fluorescent protein having an amino acid sequence selected from the group consisting of ~~SEQ ID No.3 and SEQ ID No.4~~SEQ ID NO: 3 and SEQ ID NO: 4.

Please replace the paragraph on page 31, lines 5-11, with the following amended paragraph:

The GFP gene used in the present study was contained within the plasmid pGFP (Chalfie et al., Science, (1994), 263, 802-805; GenBank accession number U17997) obtained from Clontech Laboratories Inc. (Palo Alto, Ca, USA). The gene was amplified by PCR using Pfu polymerase (Promega, Madison, WI, USA) according to recognised protocols (Saiki et al., Science, (1988), 239, 487-491). The sequences of primers used were:

GFP-1	5'-ggtacgggccgccaccatgagtaaaggagaagaactttcac	SEQ ID No.5 <u>SEQ ID NO: 5</u>
GFP-2	5'-ggtacggggttaaccggtttgtatagttcatccatg	SEQ ID No.6 <u>SEQ ID NO: 6</u>
GFP-3	5'-ggtacgggccgccaccatgggatccaaaggagaagaactttcac	SEQ ID No.7 <u>SEQ ID NO: 7</u>

Please replace the paragraph on page 32, lines 9-20, with the following amended paragraph:

The following mutants of GFP were generated in the present study: F64L-GFP, V163A-GFP, S175G-GFP, E222G-GFP, F64L-E222G-GFP, F64L-V163A-GFP, F64L-S175G-GFP, V163A-S175G-GFP, V163A-E222G-GFP, S175G-E222G-GFP, F64L-S175G-E222G-GFP, V163A-S175G-E222G-GFP, F64L-V163A-E222G-GFP, F64L-S65T-S175G-GFP, F64L-S65T-V163A-GFP. Mutants of the GFP gene (~~SEQ ID: 3~~SEQ ID NO: 3) construct within pTARGET (See Example 1) were generated using the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, Ca, USA) according to manufacturer's instructions. The sequences of primers used to generate F64L, S65T, V163A, S175G and E222G single mutants have been documented in Table 1. Multiply-mutated GFP molecules were generated through successive mutagenesis reactions. All GFP mutant sequences were verified by automated sequencing.

Please replace the heading of Table 1 on page 33, with the following amended heading:

Table 1

<u>Primer</u>	<u>Mutation</u>	<u>Sequence (5' - 3')</u>	<u>SEQ ID No: NO:</u>
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Please replace the paragraph on page 37, lines 7-11, with the following amended paragraph:

The human NFκB P65 subunit gene (GenBank Accession number: M62399) was amplified using PCR according to recognised protocols (Saiki et al., Science, (1988), 239, 487-491). The sequences of primers used were:

NFκB-1 5'-tttactcgagatggacgaactgtccccctca

~~SEQ ID No.18~~

SEQ ID NO: 18

NFκB-2 5'-tttgaagcttggagctgatctgactcagcagg

~~SEQ ID No.19~~

SEQ ID NO: 19

Please replace the paragraph on page 37, lines 12-16, with the following amended paragraph:

The P65 subunit was fused to the N terminus of GFP (~~SEQ ID No.3~~SEQ ID NO: 3) in the vector pCORON1000 (Amersham Pharmacia Biotech), under the control of a CMV promoter. This was transfected into CHO-hir cells using FuGene6 reagent (Roche) and standard transfection procedures and a stable cell line was produced containing the P65-GFP construct.